Supplementary data

Optimization of extraction parameters for the isolation of bioactive compounds from orange peel waste

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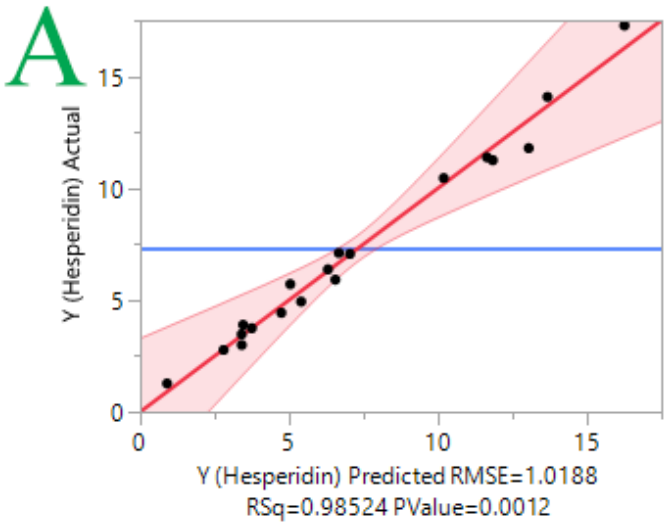
A



Summary of Fit	
RSquare	0.985237
RSquare Adj	0.9439
Root Mean Square Error	1.01877
Mean of Response	7.317
Observations (or Sum Wgts)	20

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	346.32296	24.7374	23.8342
Error	5	5.18946	1.0379	Prob > F
C. Total	19	351.51242		0.0012*

Parameter Estimates						
Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	7.1108128	1.110391	6.40	0.0014*	4.2564608	9.9651649
X1(1,4)	−1.89614	0.377017	−5.03	0.0040*	−2.865293	−0.926987
X2(1,5)	1.9766699	0.529974	3.73	0.0136*	0.6143283	3.3390115
X3(1,5)	1.9468101	0.652728	2.98	0.0307*	0.2689201	3.6247002
X4(1,5)	−0.015508	0.589295	−0.03	0.9800	−1.530339	1.4993225
X1*X1	3.9413988	0.846318	4.66	0.0055*	1.7658681	6.1169294
X1*X2	0.0864576	0.746287	0.12	0.9123	−1.831934	2.004849
X2*X2	−4.548845	0.742148	−6.13	0.0017*	−6.456597	−2.641093
X1*X3	1.0805785	1.092827	0.99	0.3682	−1.728624	3.8897808
X2*X3	2.240253	0.99324	2.26	0.0738	−0.312952	4.7934582
X3*X3	4.8811695	0.862344	5.66	0.0024*	2.664444	7.0978949
X1*X4	0.7142896	0.794112	0.90	0.4096	−1.327039	2.7556185
X2*X4	−5.610026	0.90165	−6.22	0.0016*	−7.927792	−3.29226
X3*X4	6.6135791	0.804534	8.22	0.0004*	4.5454598	8.6816984
X4*X4	−3.394639	0.935299	−3.63	0.0151*	−5.798902	−0.990376

B

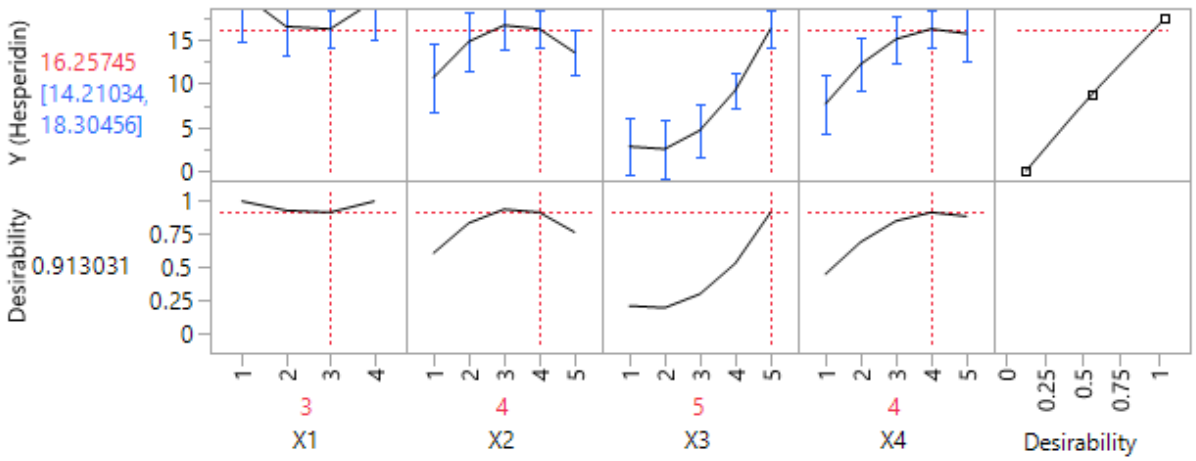
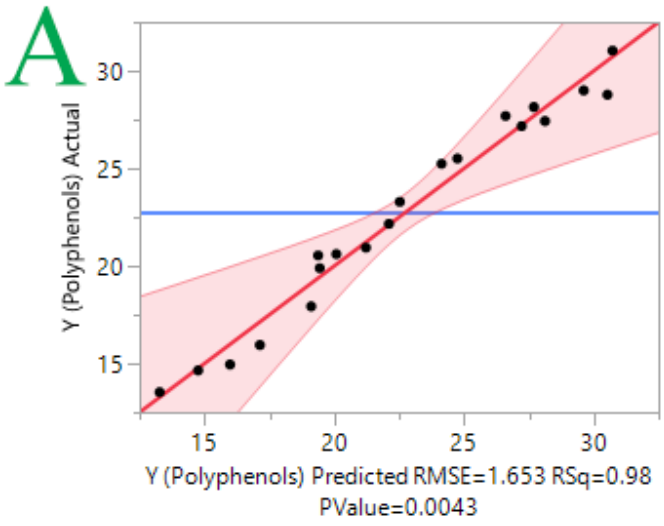


Figure S1. Plots of the actual *vs* predicted response (Hesperidin, mg/g) (plot A) and the desirability function with the extrapolation control (plot B) were created to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Statistics pertaining to the assessment of the resulting model are provided in the inset tables. Asterisks and colored values indicate the statistically significant values.

A



Summary of Fit	
RSquare	0.975403
RSquare Adj	0.906531
Root Mean Square Error	1.653015
Mean of Response	22.7225
Observations (or Sum Wgts)	20

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	541.78009	38.6986	14.1626
Error	5	13.66229	2.7325	Prob > F
C. Total	19	555.44238		0.0043*

Parameter Estimates						
Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	27.775329	1.801676	15.42	<0.0001*	23.143973	32.406685
X1(1,4)	1.8757237	0.611732	3.07	0.0279*	0.3032155	3.4482318
X2(1,5)	−0.784937	0.859914	−0.91	0.4032	−2.995418	1.425543
X3(1,5)	−0.07081	1.05909	−0.07	0.9493	−2.793287	2.6516664
X4(1,5)	−0.210393	0.956166	−0.22	0.8345	−2.668297	2.2475099
X1*X1	3.6201116	1.373202	2.64	0.0462*	0.0901836	7.1500395
X1*X2	−3.28573	1.210895	−2.71	0.0421*	−6.398435	−0.173026
X2*X2	−9.820112	1.204179	−8.16	0.0005*	−12.91555	−6.724671
X1*X3	−0.261548	1.773178	−0.15	0.8885	−4.819646	4.2965495
X2*X3	2.7525294	1.611591	1.71	0.1483	−1.390198	6.8952567
X3*X3	0.2790825	1.399204	0.20	0.8498	−3.317686	3.8758514
X1*X4	−3.629788	1.288493	−2.82	0.0372*	−6.941966	−0.317611
X2*X4	−4.442675	1.462981	−3.04	0.0289*	−8.203388	−0.681961
X3*X4	1.5522431	1.305404	1.19	0.2878	−1.803404	4.9078898
X4*X4	−4.442094	1.517579	−2.93	0.0327*	−8.343154	−0.541034

B

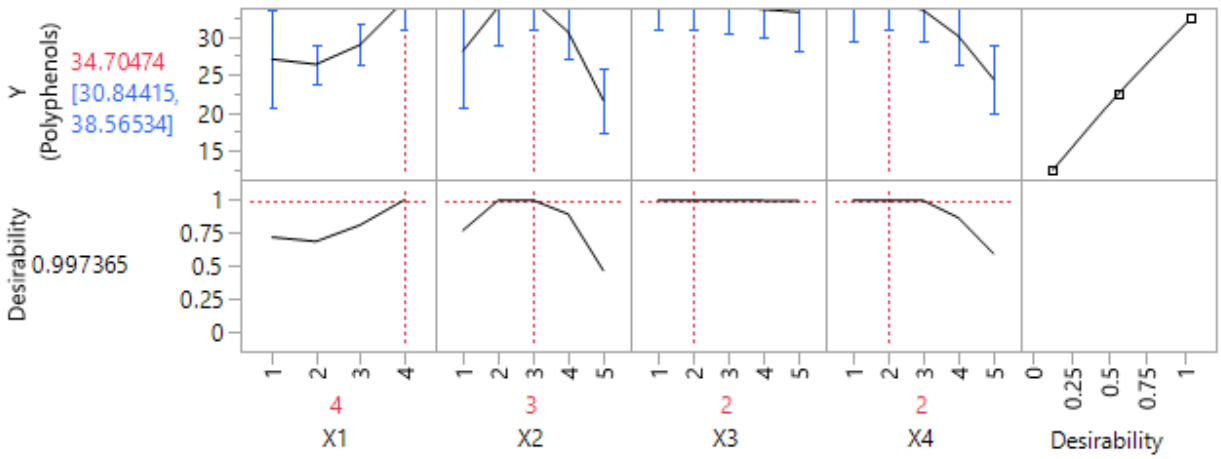
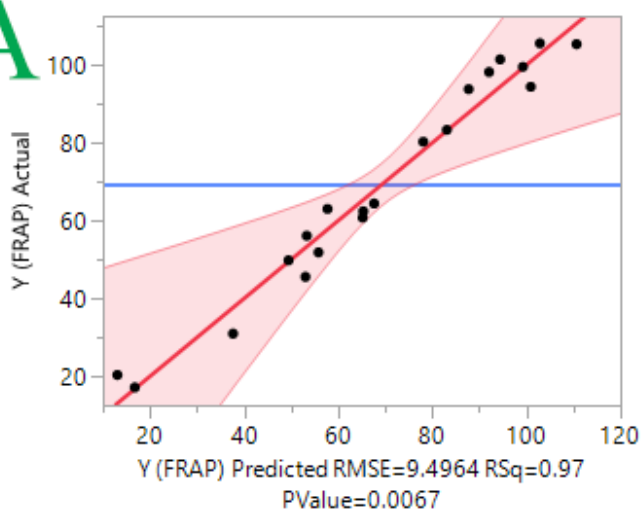


Figure S2. Plots of the actual *vs* predicted response (Polyphenols, mg GAE/g) (plot A) and the desirability function with the extrapolation control (plot B) were created to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Statistics pertaining to the assessment of the resulting model are provided in the inset tables. Asterisks and colored values indicate the statistically significant values.

A



Summary of Fit	
RSquare	0.970199
RSquare Adj	0.886754
Root Mean Square Error	9.496398
Mean of Response	69.21
Observations (or Sum Wgts)	20

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	14679.477	1048.53	11.6269
Error	5	450.908	90.18	Prob > F
C. Total	19	15130.385		0.0067*

Parameter Estimates						
Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	79.08431	10.35044	7.64	0.0006*	52.477648	105.69097
X1(1,4)	15.297623	3.514339	4.35	0.0073*	6.2637267	24.33152
X2(1,5)	−11.93429	4.940119	−2.42	0.0604	−24.63327	0.7646895
X3(1,5)	−7.352594	6.08436	−1.21	0.2809	−22.99294	8.2877518
X4(1,5)	7.0867669	5.493076	1.29	0.2534	−7.033634	21.207167
X1*X1	3.5015093	7.888903	0.44	0.6757	−16.77756	23.780581
X1*X2	−2.759848	6.956465	−0.40	0.7079	−20.64201	15.122316
X2*X2	−25.45455	6.917884	−3.68	0.0143*	−43.23754	−7.671566
X1*X3	1.4830944	10.18672	0.15	0.8899	−24.70271	27.668896
X2*X3	20.393815	9.258425	2.20	0.0788	−3.405725	44.193355
X3*X3	5.058423	8.038284	0.63	0.5568	−15.60464	25.721489
X1*X4	−6.562278	7.402261	−0.89	0.4159	−25.5904	12.465839
X2*X4	−14.96232	8.404677	−1.78	0.1352	−36.56723	6.6425893
X3*X4	17.570935	7.499408	2.34	0.0661	−1.706908	36.848778
X4*X4	0.2392214	8.718332	0.03	0.9792	−22.17196	22.650407

B

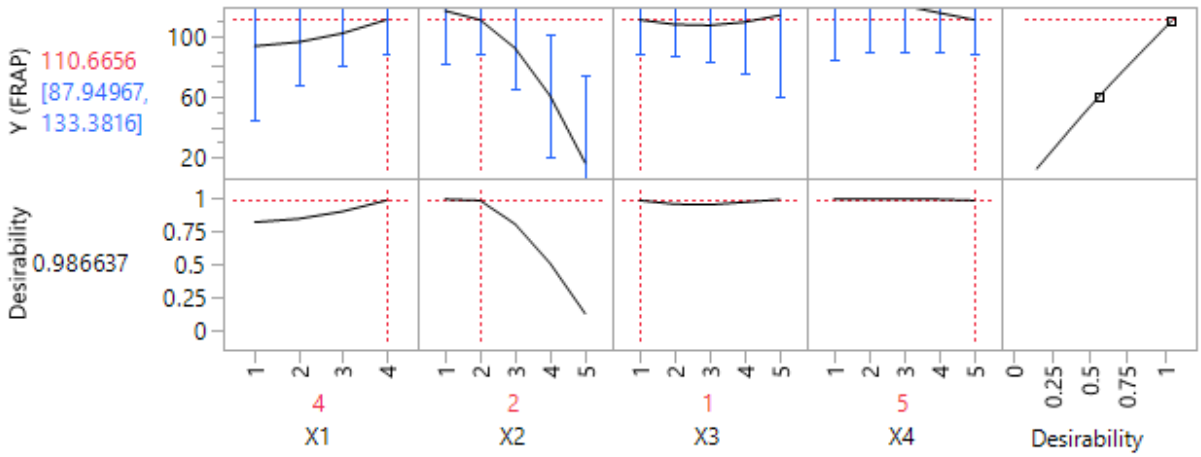
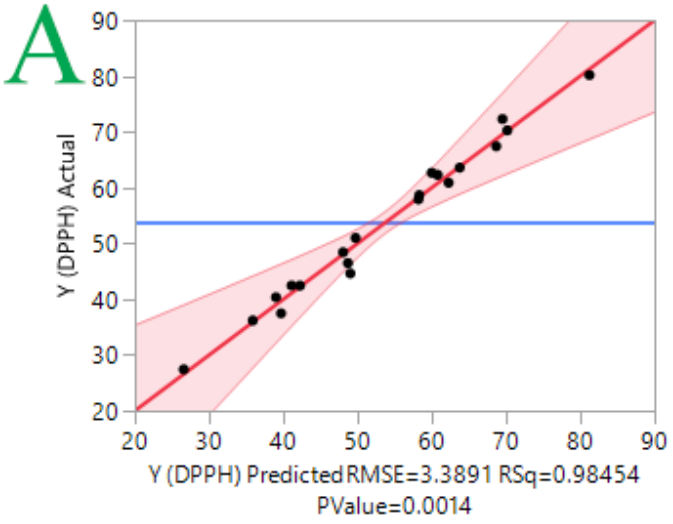


Figure S3. Plots of the actual *vs* predicted response (FRAP, μmoL AAE/g) (plot A) and the desirability function with the extrapolation control (plot B) were created to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Statistics pertaining to the assessment of the resulting model are provided in the inset tables. Asterisks and colored values indicate the statistically significant values.

A



Summary of Fit	
RSquare	0.984545
RSquare Adj	0.94127
Root Mean Square Error	3.389082
Mean of Response	53.6455
Observations (or Sum Wgts)	20

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	3658.3941	261.314	22.7509
Error	5	57.4294	11.486	Prob > F
C. Total	19	3715.8235		0.0014*

Parameter Estimates						
Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	59.46101	3.693874	16.10	<0.0001*	49.965605	68.956415
X1(1,4)	−2.341481	1.2542	−1.87	0.1209	−5.565505	0.8825424
X2(1,5)	−0.926437	1.763033	−0.53	0.6217	−5.458458	3.6055851
X3(1,5)	−2.896216	2.171391	−1.33	0.2398	−8.477954	2.6855222
X4(1,5)	−1.035877	1.960373	−0.53	0.6198	−6.075176	4.0034218
X1*X1	1.968838	2.815398	0.70	0.5155	−5.268372	9.206048
X1*X2	−12.80035	2.482629	−5.16	0.0036*	−19.18215	−6.418546
X2*X2	−18.77896	2.46886	−7.61	0.0006*	−25.12536	−12.43255
X1*X3	−5.376925	3.635445	−1.48	0.1992	−14.72213	3.9682837
X2*X3	−5.079503	3.304154	−1.54	0.1848	−13.5731	3.4140947
X3*X3	8.4363145	2.868709	2.94	0.0322*	1.0620642	15.810565
X1*X4	2.7027916	2.641724	1.02	0.3532	−4.087977	9.4935603
X2*X4	−14.75777	2.999467	−4.92	0.0044*	−22.46815	−7.047392
X3*X4	19.090989	2.676394	7.13	0.0008*	12.211098	25.97088
X4*X4	−1.622849	3.111405	−0.52	0.6242	−9.620969	6.3752717

B

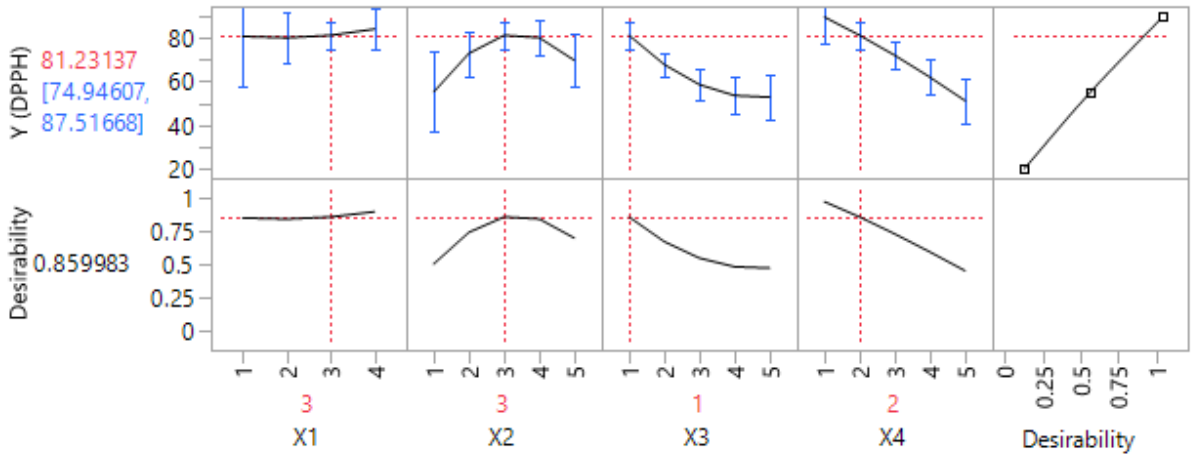
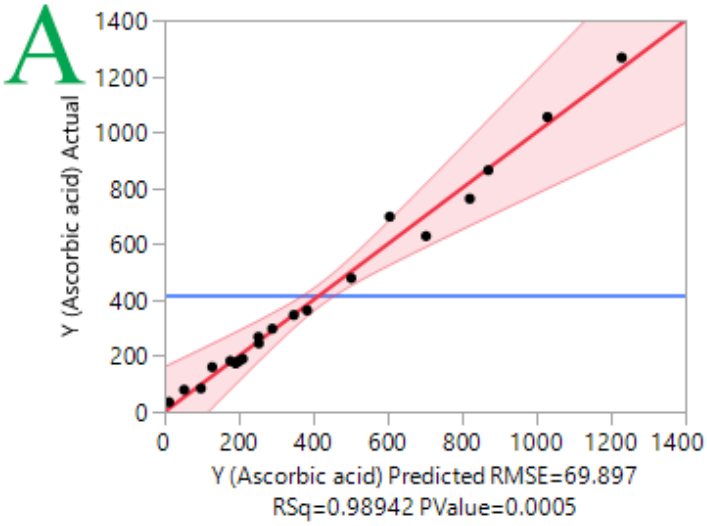


Figure S4. Plots of the actual *vs* predicted response (DPPH, μmoL DPPH/g) (plot A) and the desirability function with the extrapolation control (plot B) were created to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Statistics pertaining to the assessment of the resulting model are provided in the inset tables. Asterisks and colored values indicate the statistically significant values.

A



Summary of Fit	
RSquare	0.989418
RSquare Adj	0.959789
Root Mean Square Error	69.89677
Mean of Response	417.625
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	2284056.7	163147	33.3937
Error	5	24427.8	4886	Prob > F
C. Total	19	2308484.5		0.0005*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	402.36681	76.18284	5.28	0.0032*	206.53258	598.20104
X1(1,4)	29.86335	25.86675	1.15	0.3005	−36.62925	96.355953
X2(1,5)	446.2261	36.36098	12.27	<0.0001*	352.75721	539.69499
X3(1,5)	57.680186	44.78299	1.29	0.2541	−57.43816	172.79854
X4(1,5)	91.334928	40.43094	2.26	0.0734	−12.5961	195.26596
X1*X1	−208.1743	58.06505	−3.59	0.0158*	−357.4353	−58.91337
X1*X2	−34.07203	51.20199	−0.67	0.5352	−165.6909	97.546875
X2*X2	234.04653	50.91802	4.60	0.0059*	103.1576	364.93547
X1*X3	−185.651	74.97779	−2.48	0.0561	−378.3875	7.0855745
X2*X3	10.637487	68.14521	0.16	0.8821	−164.5354	185.81033
X3*X3	73.629086	59.16455	1.24	0.2685	−78.45822	225.71639
X1*X4	−118.8575	54.4832	−2.18	0.0810	−258.9111	21.195975
X2*X4	−20.10247	61.86133	−0.32	0.7584	−179.1221	138.91713
X3*X4	−85.18794	55.19824	−1.54	0.1834	−227.0795	56.703648
X4*X4	−61.47218	64.16994	−0.96	0.3821	−226.4262	103.48189

B

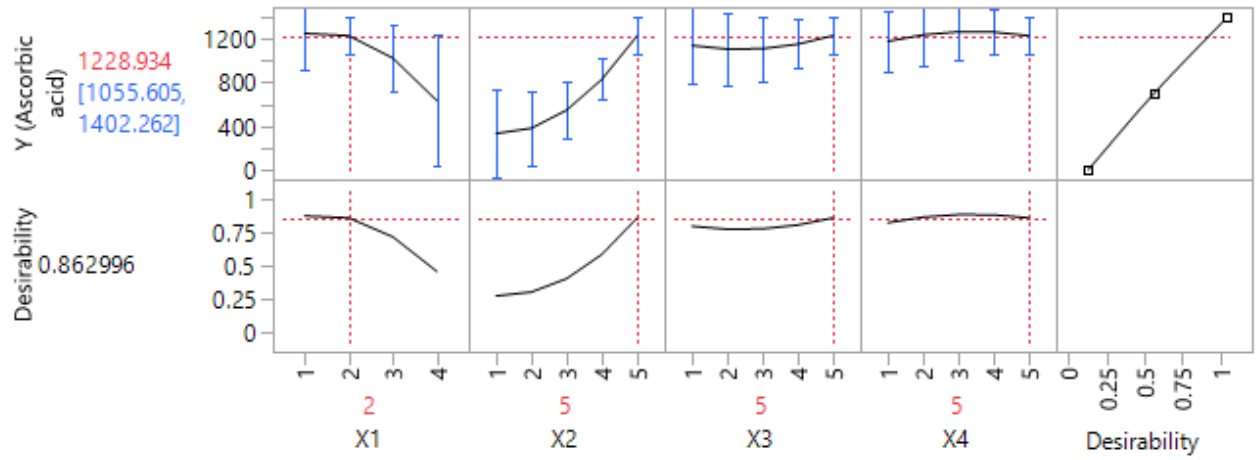
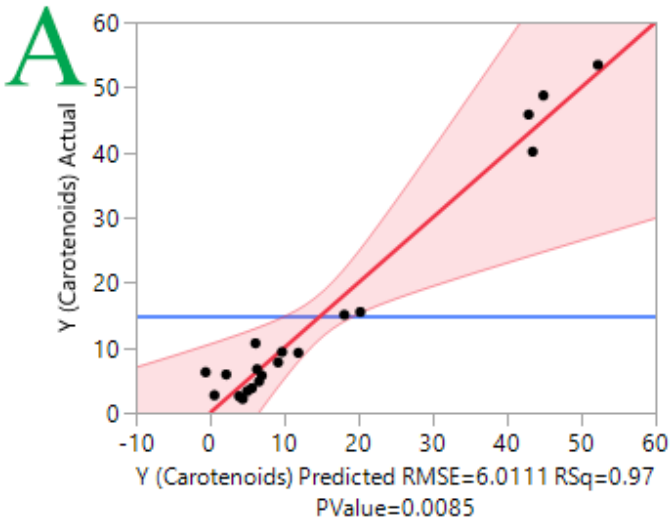


Figure S5. Plots of the actual *vs* predicted response (ascorbic acid, mg/100 g) (plot A) and the desirability function with the extrapolation control (plot B) were created to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Statistics pertaining to the assessment of the resulting model are provided in the inset tables. Asterisks and colored values indicate the statistically significant values.

A



Summary of Fit	
RSquare	0.967071
RSquare Adj	0.874872
Root Mean Square Error	6.01112
Mean of Response	14.9775
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	5305.9957	379.000	10.4889
Error	5	180.6678	36.134	Prob > F
C. Total	19	5486.6636		0.0085*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	10.066816	6.551722	1.54	0.1850	-6.774922	26.908555
X1(1,4)	1.4621533	2.22454	0.66	0.5400	-4.256209	7.1805153
X2(1,5)	19.487773	3.127044	6.23	0.0016*	11.449451	27.526095
X3(1,5)	-0.133939	3.851336	-0.03	0.9736	-10.03411	9.7662356
X4(1,5)	2.1908124	3.477059	0.63	0.5563	-6.747253	11.128878
X1*X1	-8.10302	4.993593	-1.62	0.1656	-20.93946	4.7334193
X1*X2	-0.349424	4.40337	-0.08	0.9398	-11.66865	10.969799
X2*X2	20.879643	4.378948	4.77	0.0050*	9.6231982	32.136088
X1*X3	-2.830121	6.448088	-0.44	0.6790	-19.40546	13.745217
X2*X3	2.9490327	5.860486	0.50	0.6362	-12.11583	18.013893
X3*X3	-3.338766	5.088149	-0.66	0.5407	-16.41827	9.7407379
X1*X4	-1.391822	4.685554	-0.30	0.7784	-13.43642	10.652777
X2*X4	8.4514158	5.320073	1.59	0.1730	-5.224266	22.127098
X3*X4	-9.013462	4.747047	-1.90	0.1160	-21.21614	3.1892104
X4*X4	0.5615244	5.518613	0.10	0.9229	-13.62452	14.74757

B

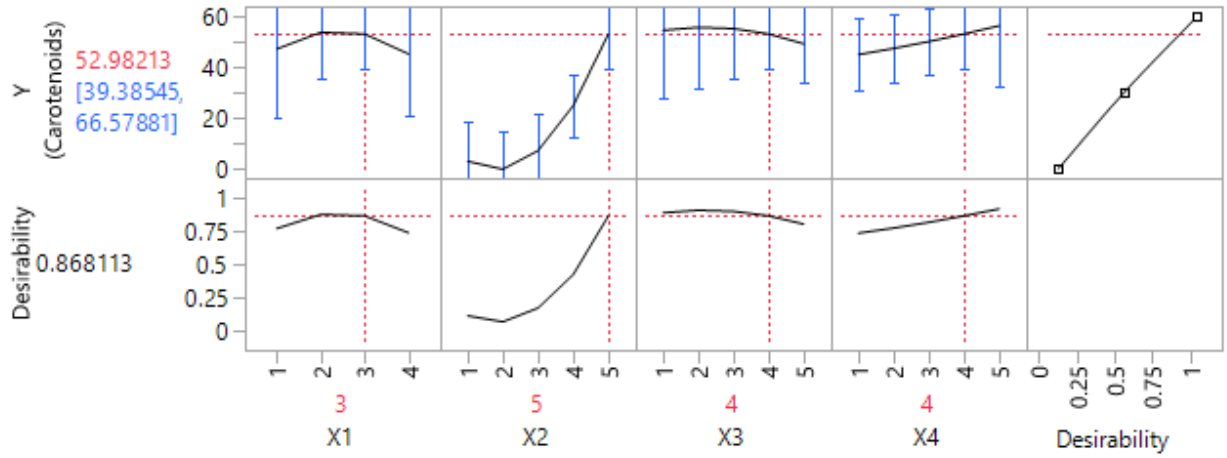


Figure S6. Plots of the actual *vs* predicted response (Carotenoids, μg CtE/g) (plot A) and the desirability function with the extrapolation control (plot B) were created to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Statistics pertaining to the assessment of the resulting model are provided in the inset tables. Asterisks and colored values indicate the statistically significant values.

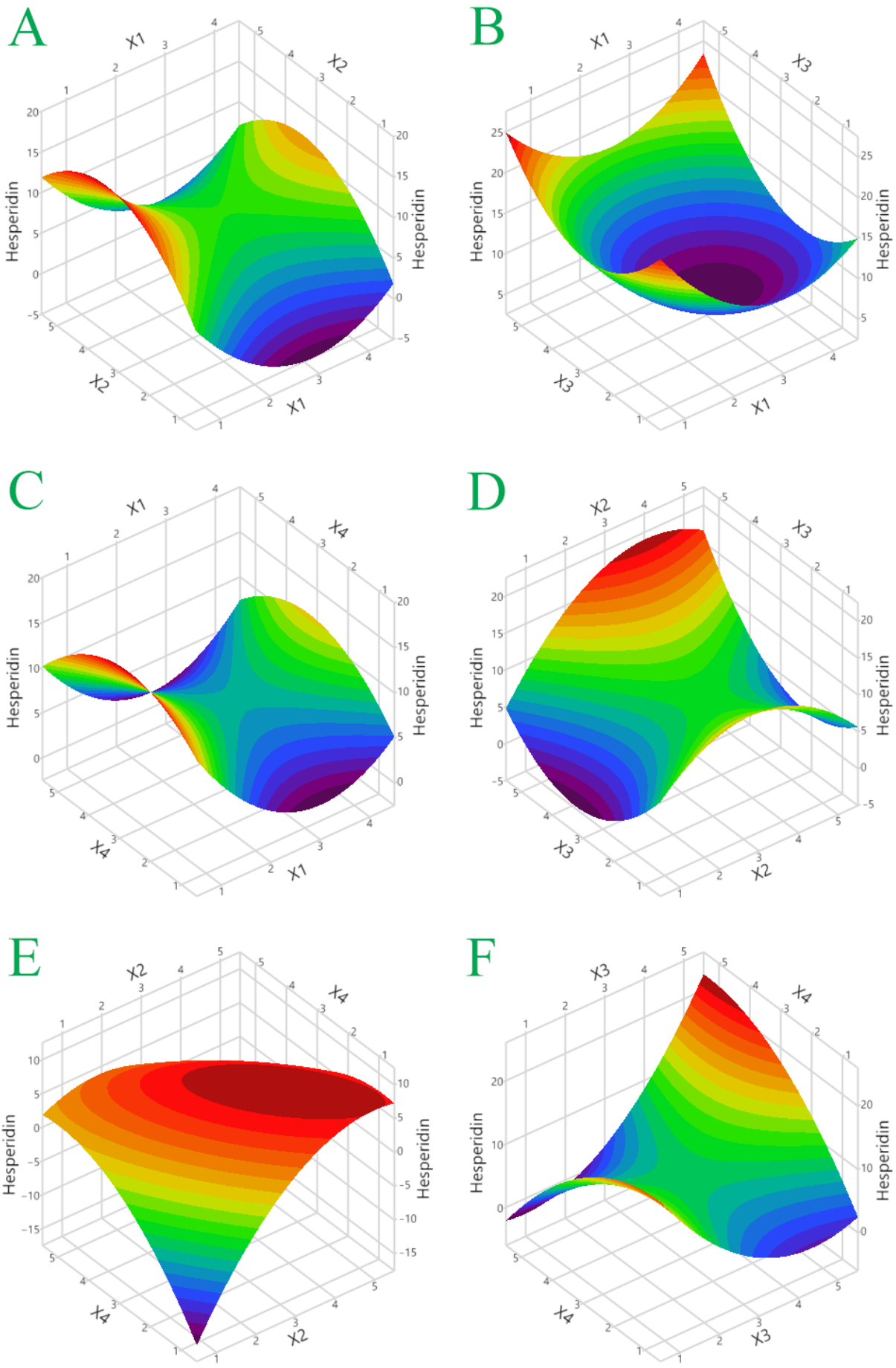


Figure S7. 3D graphs depicting the effect of the process variables considered in the response (Hesperidin, mg/g), to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

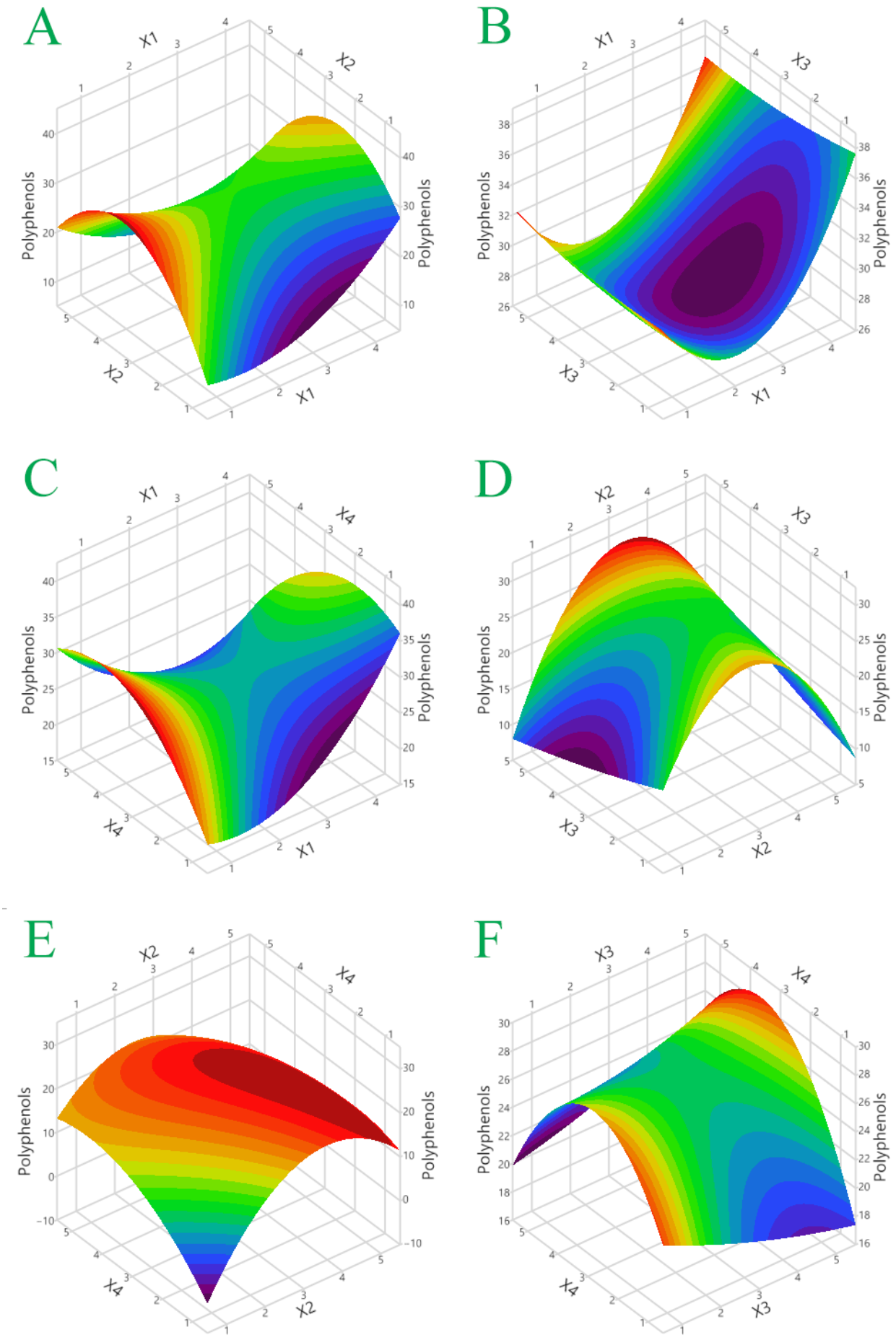


Figure S8. 3D graphs depicting the effect of the process variables considered in the response (Polyphenols, mg GAE/g), to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

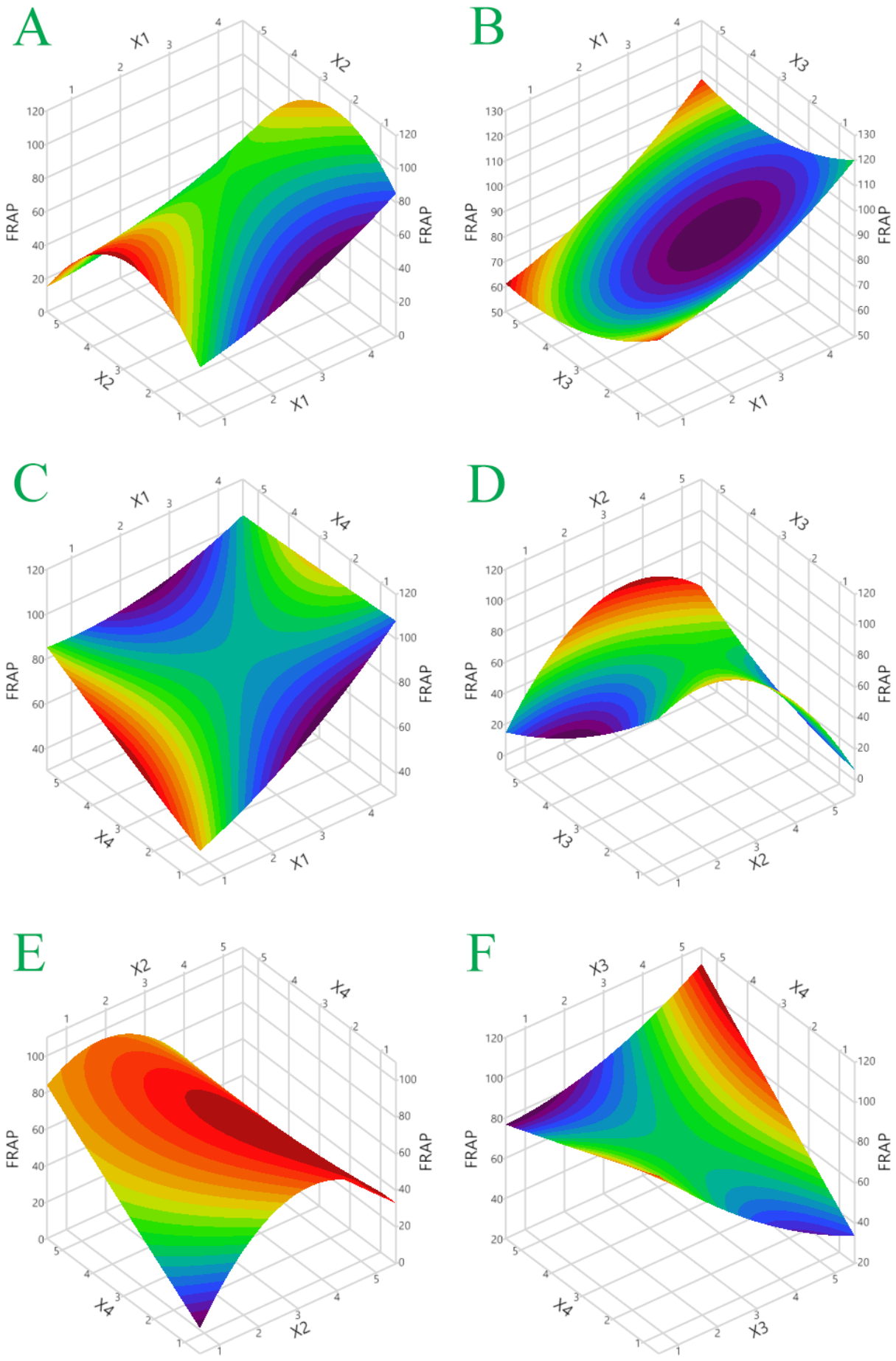


Figure S9. 3D graphs depicting the effect of the process variables considered in the response (FRAP, μmoL AAE/g), to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

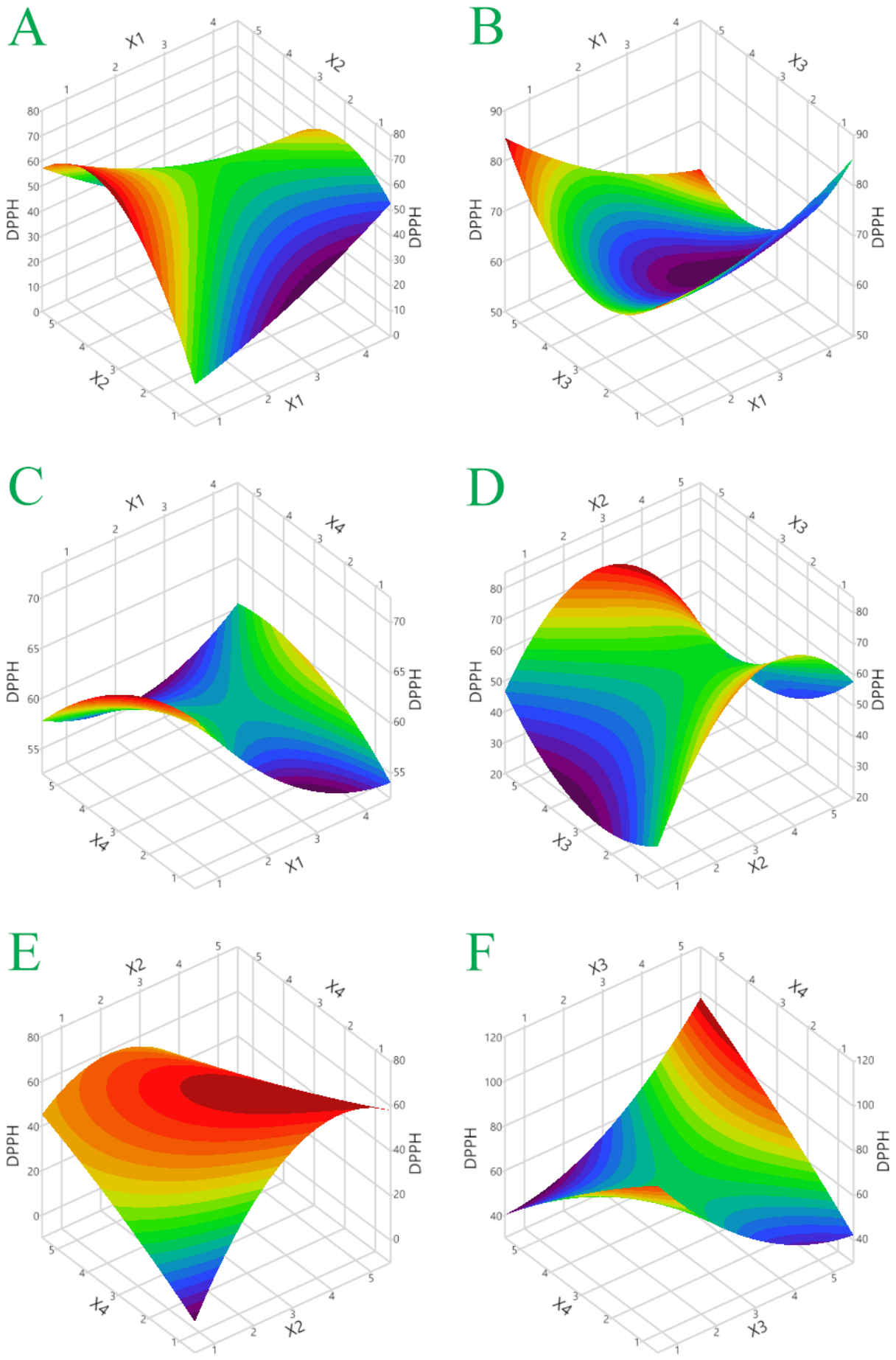


Figure S10. 3D graphs depicting the effect of the process variables considered in the response (DPPH, μmoL DPPH/g), to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

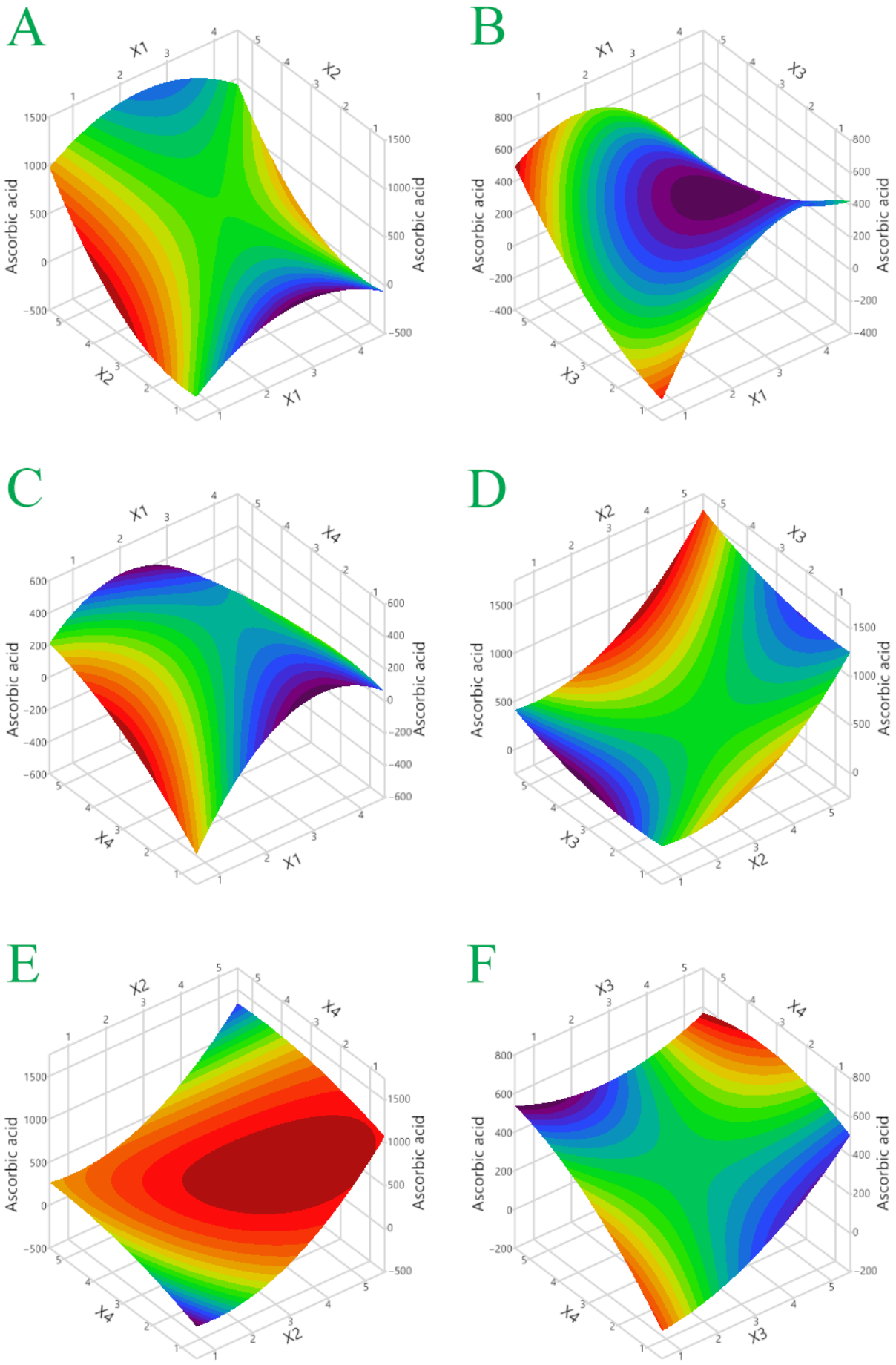


Figure S11. 3D graphs depicting the effect of the process variables considered in the response (ascorbic acid, mg/100 g), to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

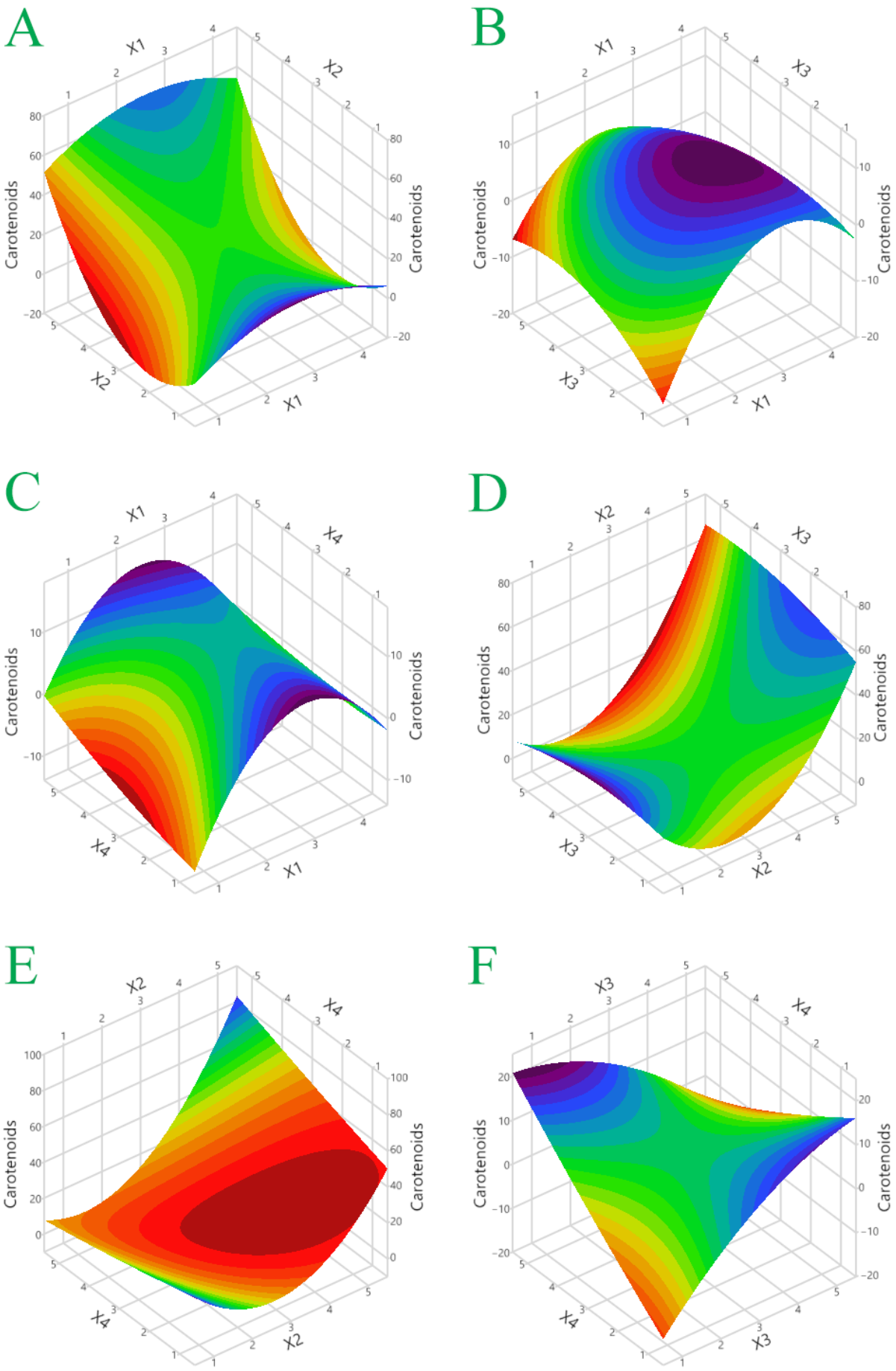


Figure S12. 3D graphs depicting the effect of the process variables considered in the response (Carotenoids, μg CtE/g), to optimize the extraction of the orange peel, using various extraction techniques and hydroethanolic solutions. Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

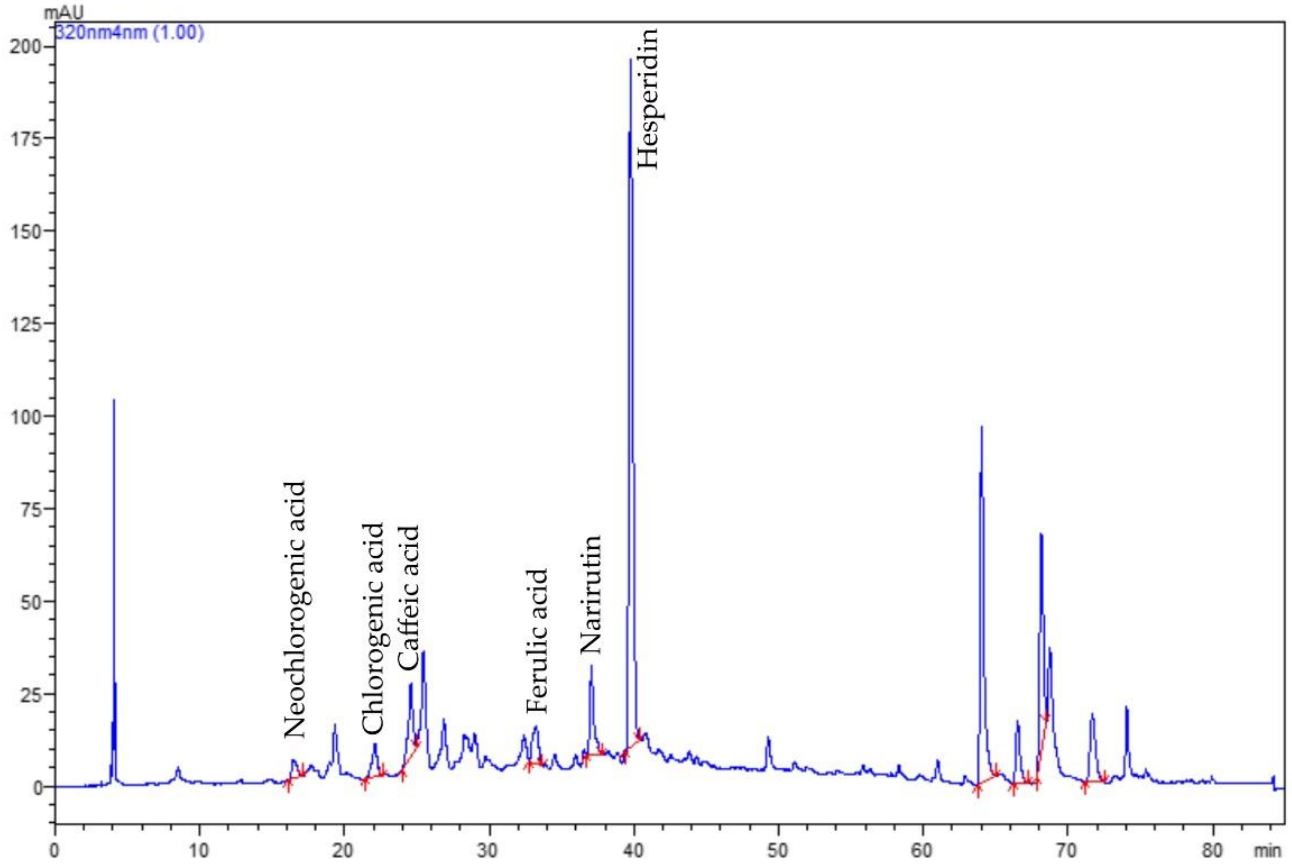


Figure S13. Representative chromatogram of an orange peel extract, at 320 nm, depicting the identified polyphenols.

Table S1. Coded values of the four independent variables investigated and the actual concentration of the phenolic compounds; Results are expressed in mg/g dw; ND: not detected.

Design point	Independent Variables				Caffeic acid	Ferulic acid	Narirutin	Neochlorogenic acid	Chlorogenic acid
	X_1	X_2	X_3	X_4					
1	1	1	4	3	0.09	0.06	1.10	ND	0.09
2	1	2	5	1	0.21	0.08	0.86	0.20	0.61
3	2	3	3	1	0.22	0.07	1.12	0.15	0.60
4	1	4	3	2	0.17	0.07	1.34	0.05	0.35
5	1	5	1	4	0.12	0.15	0.83	0.21	0.55
6	4	1	5	2	0.13	0.16	0.75	0.25	0.58
7	2	2	4	2	0.21	0.10	0.94	0.20	0.65
8	1	3	2	5	0.22	0.07	1.19	0.15	0.55
9	2	4	1	3	0.18	0.07	1.41	0.06	0.39
10	2	5	5	5	0.09	0.06	1.28	ND	0.05
11	2	1	2	4	0.13	0.16	0.89	0.19	0.58
12	3	2	2	3	0.24	0.08	1.02	0.22	0.71
13	4	3	4	4	0.23	0.08	1.39	0.13	0.54
14	3	4	5	4	0.18	0.08	1.53	0.04	0.39
15	3	5	4	1	0.07	0.04	0.94	ND	0.05
16	3	1	3	5	0.18	0.13	0.85	0.25	0.62
17	4	2	1	5	0.23	0.08	0.99	0.21	0.67
18	3	3	1	2	0.23	0.08	1.15	0.14	0.67
19	4	4	2	1	0.12	0.08	1.31	0.02	0.44
20	4	5	3	3	0.08	0.06	1.13	ND	0.06